Rhizoid Formation in Megagametophytes of Marsilea in Response to Growth Substances

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In an earlier paper by Bloom (1962), dealing with rhizoid formation in unfertilized megagametophytes of *Marsilea*, it was suggested that rhizoid formation is a geotropic response involving an endogenous auxin. It was suggested that the auxin becomes concentrated in the ventral region of the gametophyte plant as a result of gravitational force. As a result of a series of experiments conducted in our laboratories, further evidence has been gathered to confirm the roles of auxins and gravitational forces in determining rhizoid formation.

Maravolo and Voth (1966) have reported that gemmalings of *Marchantia* normally form ventral rhizoids on new tissue, but that when exogenous auxins are applied and reach a threshold concentration on the dorsal surface, rhizoids form there as well. The number of rhizoids formed was reported to be proportional to the concentration of the exogenous auxin. Miller and Miller (1964) reported effects of auxins on cell enlargement in fern gametophytes of *Onoclea sensibilis*, but did not report any observations of unusual effects on rhizoid formation. Kelley and Postlethwait (1962) studied the effects of the plant growth substance 2-chloroethyltrimethyl-ammonium chloride on gametophytes of *Pteridium aquilinum* and noted an effect on the time sequence of rhizoid production, but did not report any unusual effect on the position of the rhizoids formed.

If auxins are indeed involved in rhizoid formation in the megagametophytes of *Marsilea* and if a higher concentration of auxin at the ventral sites is a response to gravitational force, certain predictions can be made concerning the effect of the application of exogenous auxins to the megagametophytes. In diverse processes auxins are generally stimulatory at certain concentrations and inhibitory at higher concentrations (Leopold, 1964). It could be predicted that rhizoid formation would be inhibited at high concentrations of exogenous auxins and stimulated at low concentrations. An additive effect could be expected if exogenous auxins are applied at very low concentrations.

To test the above hypotheses the following experiments were conducted. Mineral nutrient solutions were prepared using Hoagland's No. 2 solution as given by Hoagland and Arnon (1938), with appropriate micronutrients added. Separate solutions were prepared by adding indole-3-acetic acid, napthalene acetic acid, or gibberellic acid in amounts to provide concentrations by weight of one part in 10,000 (10^{-4}), 100,000 (10^{-5}), 1,000,000 (10^{-6}), and 10,000,000 (10^{-7}). Plain agar was added at a concentration of 1.5% to each solution and the solutions were autoclaved and poured into sterile 100 × 15 mm plastic Petri dishes.

Sporocarps of *Marsilea mucronata* were hydrated in deionized water. As soon as the megaspores were free they were transferred to the surface of the agar plates. Organic materials other than agar were not included in the media since the mega-

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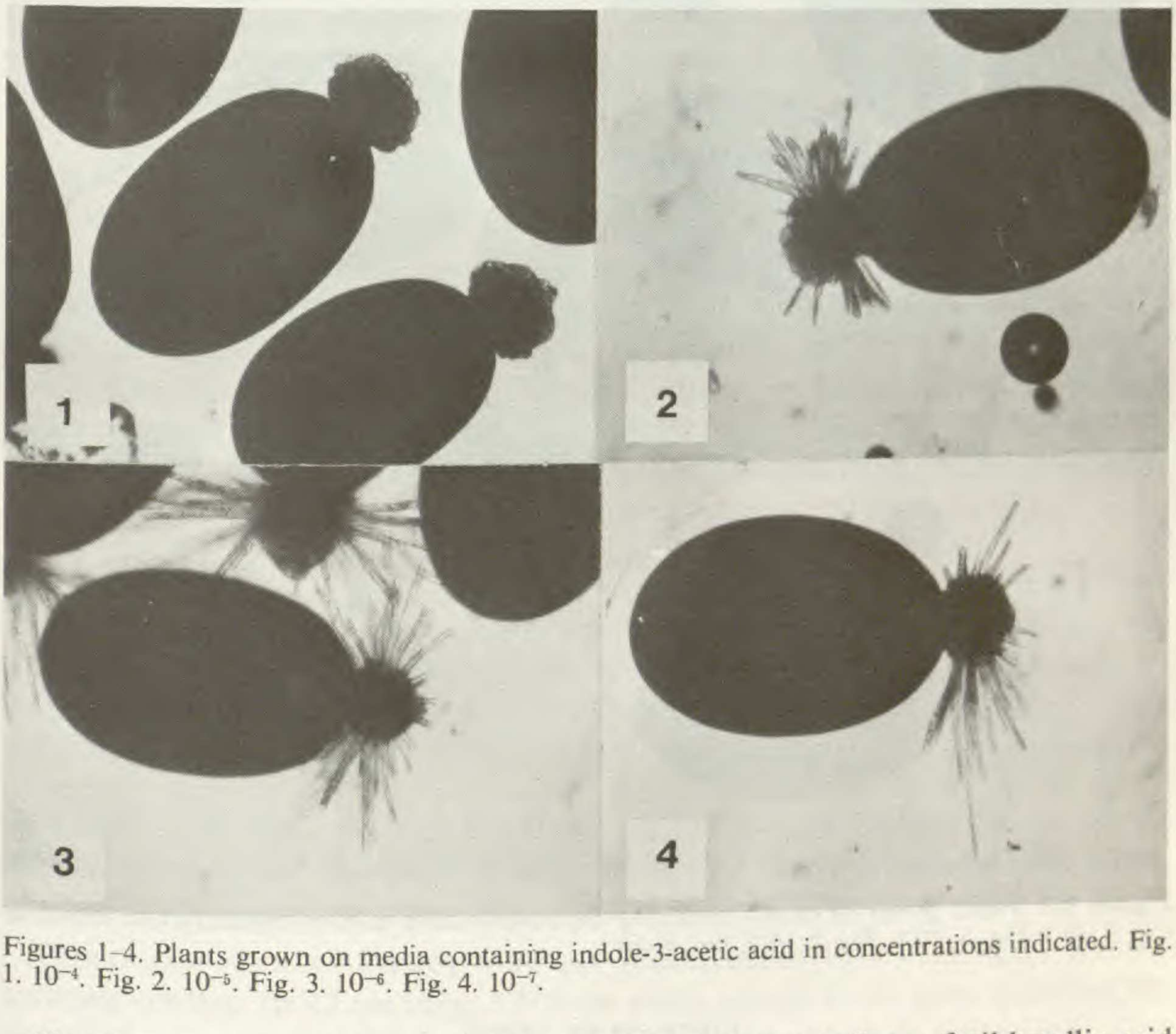
spores contain a considerable amount of food reserve in the form of starch. After the megaspores had been transferred to the agar surface, the plates were changed from a horizontal to a vertical position and marked to indicate the ventral position. One-half of the plates were maintained in constant illumination from cool white fluorescent lights at an intensity of approximately 200 ft-c. The remaining plates were covered with aluminum foil to exclude the light. The laboratory was maintained at a temperature of 24°C during the experiment. At the end of a 49-hour growth period the plates were examined and representative plants were photographed. The megagametophytes were photographed *in situ* after placing a drop of water on the plants and covering them with a cover glass. The pictures were taken with a Bausch & Lomb photobinocular microscope at 80 × with Polaroid 3000 film using a 1.7 filter, iris closed, stop 5 on the illuminator, at 1/5 second.



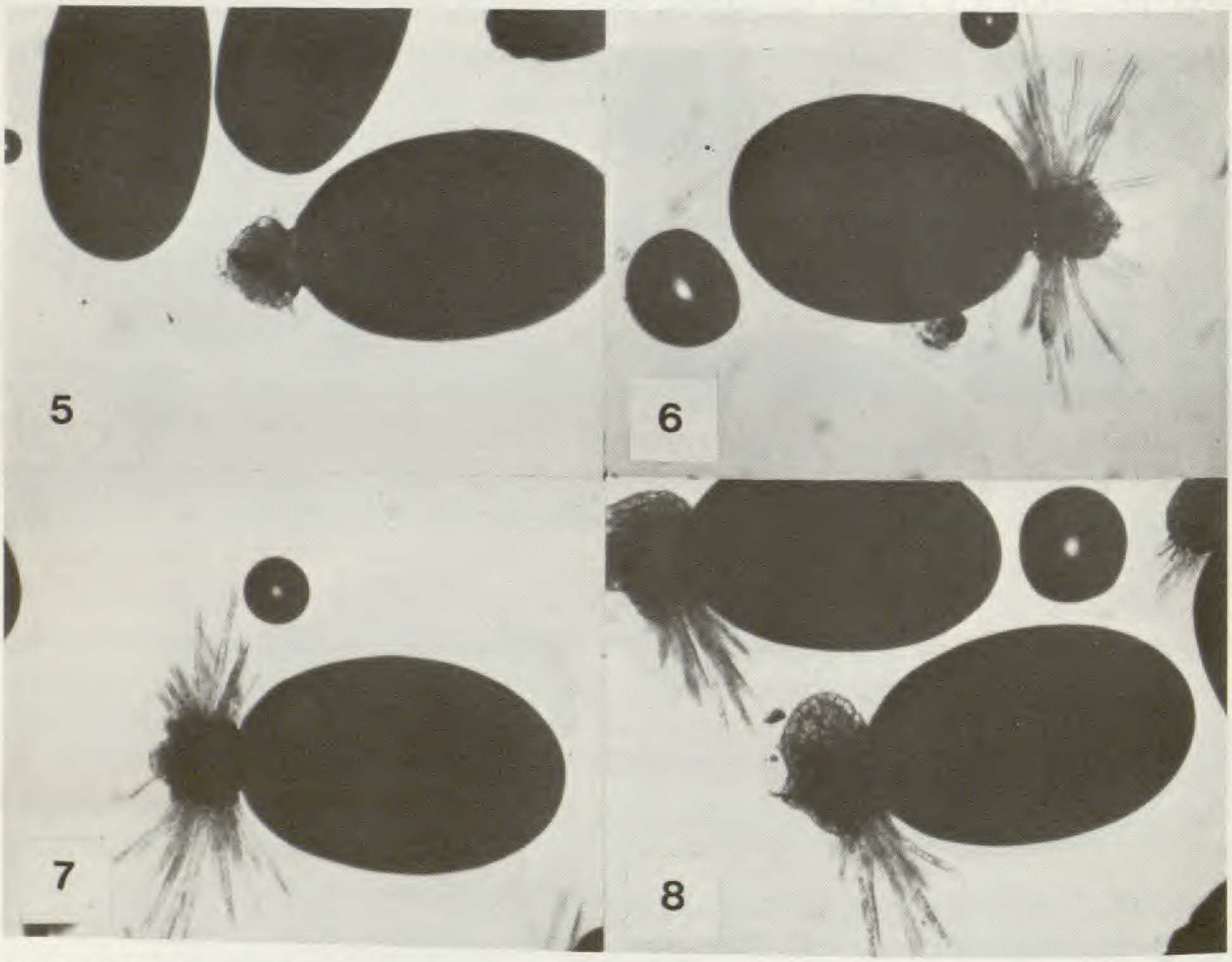
Figures 1–4. Plants grown on media containing indole-3-acetic acid in concentrations indicated. Fig. 1. 10^{-4}. Fig. 2. 10^{-5}. Fig. 3. 10^{-6}. Fig. 4. 10^{-7}.

Plants grown on media containing the various concentrations of gibberellic acid were all similar. The rhizoids developed in the ventral portion of the megagametophytes, as also occurred in the controls. Little variation could be detected regardless of the concentration of gibberellic acid. These plants were characteristically a deeper green than the other experimental plants.

Figures 1–4 illustrate the results with plants grown in the dark and treated with concentrations of indole-3-acetic acid as noted in the legend. Concentrations of 10^{-4} were inhibitory (*Fig. 1*); concentrations of 10^{-5} were stimulatory (*Fig. 2*), producing rhizoids on both dorsal and ventral surfaces, but less effectively than concentrations of 10^{-6} (*Fig. 3*); concentrations of 10^{-7} were mildly stimulatory, as indicated by the short rhizoids on the dorsal surface and additive to endogenous auxins as shown by the longer, more numerous ventral rhizoids (*Fig. 4*).

Figures 5–8 illustrate the results with plants grown in the dark and treated with concentrations of napthalene acetic acid as noted in the legend. Concentrations of 10^{-4} were inhibitory (*Fig. 5*); concentrations of 10^{-5} (*Fig. 6*) and 10^{-6} (*Fig. 7*) were stimulatory; concentrations of 10^{-7} were not stimulatory, as indicated by the absence of rhizoids on the dorsal surface (*Fig. 8*).

Plants grown in the light were generally larger and contained more chlorophyll than the plants grown in the dark. It was not possible at this time to determine whether the increased growth in the light was due to photosynthetic activity or whether light caused an increase in auxin production.



Figures 5–8. Plants grown on media containing napthalene acetic acid in concentrations indicated Fig. 5. 10^{-4}. Fig. 6. 10^{-5}. Fig. 7. 10^{-6}. Fig. 8. 10^{-7}.

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